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14. ABSTRACT Nanotechnology has the potential to develop silicon-based arrays for sensing biomarkers associated with breast cancer.. Until recently, breast cancer research has focused on a small number of genes or proteins as primary biomarkers. In order to develop patient-specific therapy, tailored for each individual, parallel detection of a large number (~103-104) biomarkers may be required. The experience of the semiconductor industry in developing large scale integrated circuits at very low cost can lead to similar breakthroughs in array sensors for biomolecules of interest to the breast cancer community. Nanotechnology can meet the need for high throughput, sensitive methods for rapidly recording biomarker profiles of tumors in individual patients. We report results on the development of arrays of conductance sensors of bio-functionalized silicon nanowires. For nanoscale wires, such as those used in this study, the change is primarily due to the contribution of surface states to the conductance. The fractional change is greatest for the smallest sensors, due to the increased surface-to-volume ratio. The fabrication of arrays of conductance based sensors has now been done, and the nanosensors have been characterized using model systems. The utility of these newly fabricated sensors to actual clinical breast cancer practice now remains the main goal of our project.					
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INTRODUCTION

In this annual report we describe the results of a research effort into the development of nanosensors for breast cancer biomarkers. In our last annual report, we had described the discovery of a gating phenomenon that had the potential for greatly improving the reproducibility and reliability of individual nanosensors, and the potential for building a nanosensor array, leading to cheaper and faster methods of detecting biomolecular markers.

Broadly, the research has started out with several specific aims:

1. Design of a **nanomechanical** cantilever based sensor for biomolecular recognition, using Finite-Element simulation.
2. **Fabrication** of Biofunctionalized Nanoscale Sensors capable of detecting targeted molecules at a concentration of less than 1 ng/ml.
3. Demonstration of the ability to make an **array of nanosensors**, capable of performing highly parallel studies on selected biomarkers, based on the field effect gating principle.
4. Characterization of functionalized nanosensors for selected **breast cancer markers**, and comparing with existing immunohistochemical and Fluorescence *in situ* hybridization (FISH) techniques on well established biomarkers such as Her-2/*neu*, estrogen and progesterone hormone receptors in tumor tissue, and selected mucin antigens in blood.

Our most notable success this year has been to further develop the very exciting **gating principle**. We report here the **fabrication of an array of nanosensors**, showing sensitive to model systems. We have **characterized** the array using a model system. We have now to establish that this sensitivity extends to breast cancer biomarkers and are waiting for approval from the DOD Office of Research Protectons concerning human. As soon as approval is granted studies on samples derived from patients will be initiated. Combined with studies on tissue samples from patients, we feel that we will be able to meet all the specific aims of the IDEA award.

BODY OF REPORT

We report results on the sensitivity of nanoscale sensors by the measurement of conductance change of bio-functionalized nanowires. We also report the fabrication of an array of nanosensors, along with a field effect gate and characterization studies. Last year, we reported simulation studies of the mechanical response of the sensors. Our results show that electrical measurements are very promising and provide a simple, effective and potentially inexpensive method for biomarker sensing. The change in conductance is primarily due to the contribution of surface states to the conductance, which for larger sensors is dominated by volume effects. The fractional change is greatest for the smallest sensors, due to the increased surface-to-volume ratio.

Fabrication: Our silicon nanowires are fabricated from Silicon-On-Insulator wafer by electron beam lithography, which provides highly controllable nanowire sensors in comparison to other nanoelectronic approaches. We detect ultra-sensitive conductance change at

nanoampere-level currents in functionalized nanowires with a silane-modified surface. The finest nanosensors studied are 200 nm wide and are among the smallest lithographically-controlled nanosensors that have fabricated for biomarker sensing. We also report on studies on slightly larger 300 nm wide sensors, which have better saturation properties suitable for large scale production, and on control studies on 10 μ wide silicon strips.

Key Research Accomplishments

We list here the accomplishments so far:

1) We have used simulations to **characterize and develop nanoscale cantilever sensors**. Simulations conducted in the presence of water show that hydrodynamic effects have to be taken into account in order to properly characterize the sensitivity of these sensors. The refined theoretical model has been published in the prestigious journal *Physical Review Letters*. At the same time, we have performed electrical conductance measurements on nanowires in order to assess the sensitivity.

2) We have successfully used electron beam lithography to **fabricate nanosensors** and have **functionalized** them using silanization protocols. Some of the nanomechanical structures our collaboration has developed is among the smallest and highest frequency nanomechanical resonator ever constructed. In performing conductance measurements on the structures, we have discovered a very interesting **gating effect** that can be used to enhance the sensitivity of the nanosensors.

3) We have successfully **fabricated an array of silicon nanosensors** using electron beam lithography to fabricate nanosensors and have functionalized them using silanization protocols. The preliminary array has a total of 92 nanosensors in an area that is smaller than a single "pixel area" commonly used in optical gene chips. Thus electrical nanosensors can potentially be packed $\sim 10^2$ times more densely than optical gene chips, and thus potentially to greater degree of parallelism.

The task of using the developed nanosensors, both single element and arrays, for measuring the sensitivity and specificity for breast cancer biomarkers from patients awaits approval from DOD Office of Research Protections. [Approval of our protocol from the Institutional Review Board at our institution has already been granted.]

Reportable Outcomes

We have used simulations to characterize and develop several types of nanosensors. Figure 1 shows a schematic of the electron-beam lithography method used to fabricate nanowire

sensors attached to gold pads for electrical measurements. The figure shows a single element device that was reported in our previous annual report and some of the description is repeated here for clarity. Briefly, a single silicon nanosensor is fabricated on a Silicon-on-insulator (SOI) platform using electron—beam lithography. e-beam lithography provides the ability to fabricate < 100 nm scale structures on a scale much smaller than optical or UV lithographic methods commonly used in the micro-electromechanical systems (MEMS). The silicon nanowire is supported by an insulating silicon oxide bridge. In this report, the fabricated nanowires range in cross-section from $200\text{ nm} \times 100\text{ nm} \times 10\text{ }\mu\text{m}$ to $300\text{ nm} \times 100\text{ nm} \times 10\text{ }\mu\text{m}$ and larger. Shown in Figure 1 is an electron microscope image of several single nanosensors, showing the placement of gate electrodes on the side. The gate electrodes were used to demonstrate that the nanosensors could be operated in a manner analogous to a Field-effect transistor (FET). The system can be thought of as a BioFET, with all the advantages of amplification, reproducibility, sensitivity that FETs provide. The engineering of our device consists of two fundamental steps: fabrication and functionalization. The silicon nanowire along with the side gates and the electrodes are fabricated by standard electron beam lithography and surface nanomachining. The starting SOI wafer has a device layer thickness of 230 nm and oxide layer thickness of 370 nm with a starting device-layer volume resistivity of 10-20 ohm-cm. The device-layer resistivity is further controlled by doping the wafer by ion implantation of boron with a concentration of 1×10^{18} /cc by ion implantation.

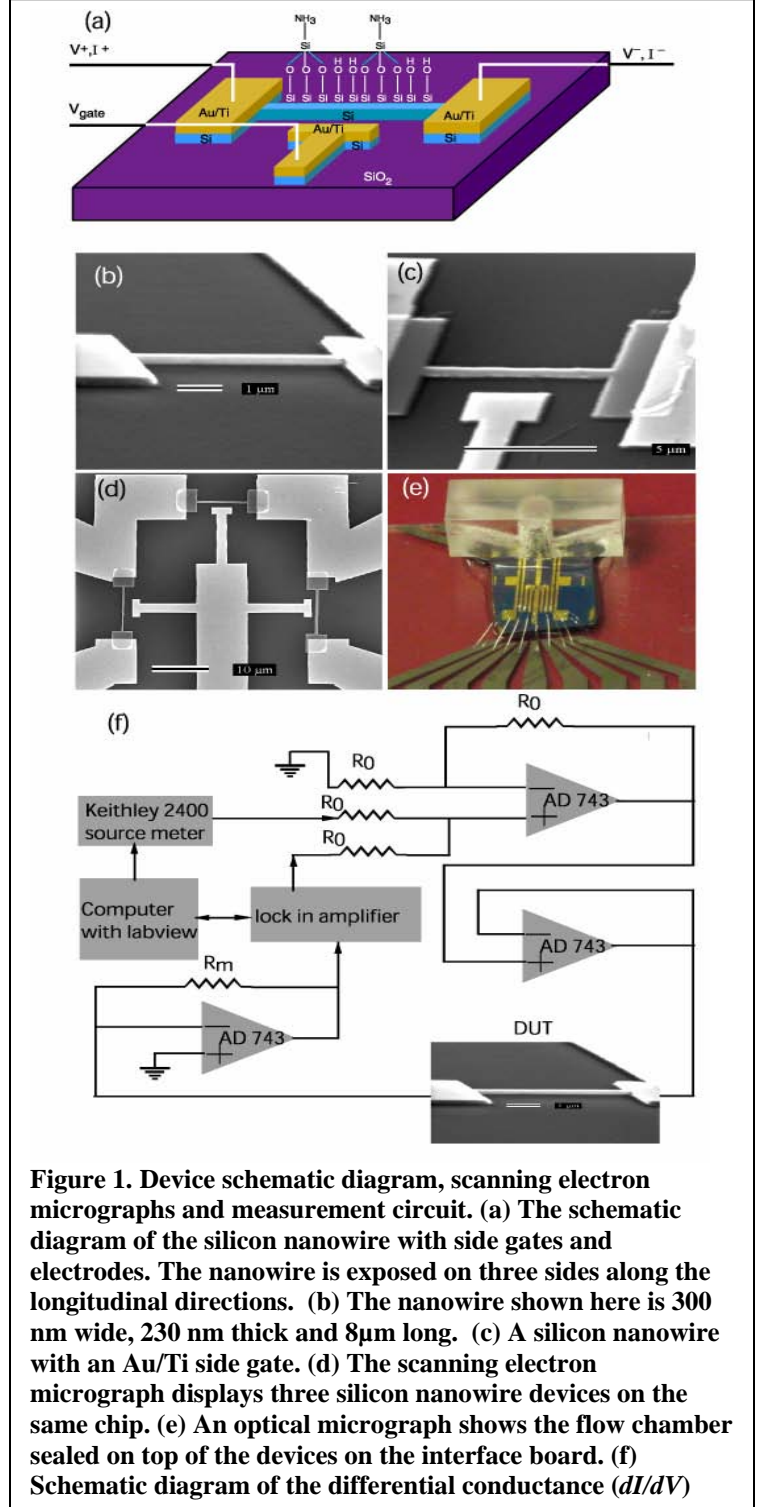


Figure 1. Device schematic diagram, scanning electron micrographs and measurement circuit. (a) The schematic diagram of the silicon nanowire with side gates and electrodes. The nanowire is exposed on three sides along the longitudinal directions. (b) The nanowire shown here is 300 nm wide, 230 nm thick and 8 μm long. (c) A silicon nanowire with an Au/Ti side gate. (d) The scanning electron micrograph displays three silicon nanowire devices on the same chip. (e) An optical micrograph shows the flow chamber sealed on top of the devices on the interface board. (f) Schematic diagram of the differential conductance (dI/dV)

After patterning the nanowires and the electrodes in separate steps with separate masks, the structure is etched out with an anisotropic reactive-ion etch (RIE). In this report, we have performed extensive measurements of the field-effect principle, and also succeeded in fabricating arrays of nanosensors, described below.

Nanomechanical sensors: Figure 2 shows how the presence of hydrodynamic effects can change the nanomechanical properties of such nanoscale resonators.

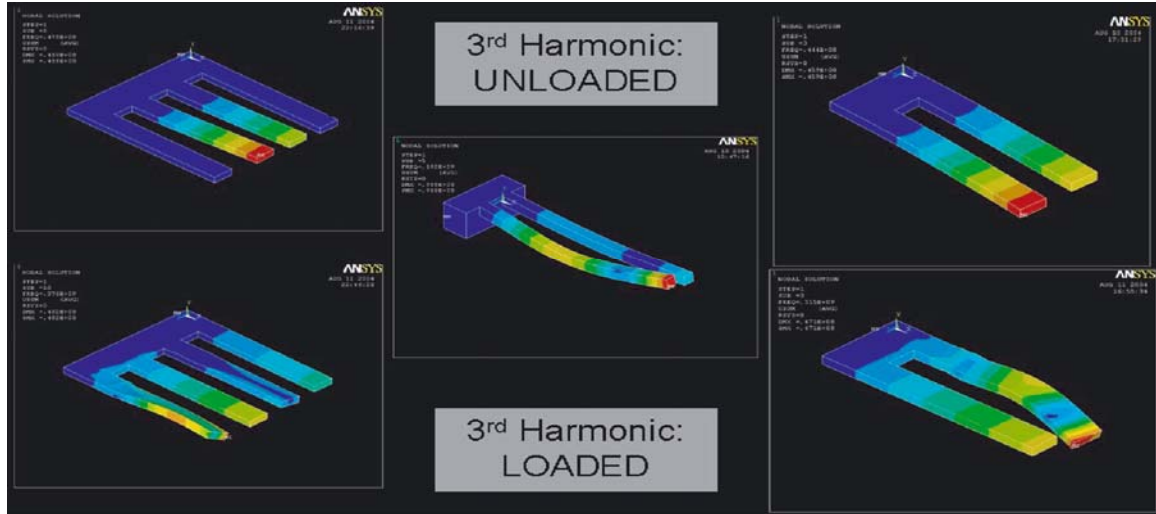


Figure 2. Finite element simulations of the nanomechanical properties of the nanoscale tuning forks. The color indicates the distribution of the strain field in a the dominant normal mode.

When stress (load due to binding of biomarkers, for example) is applied, the response also depends on the hydrodynamic properties of the surrounding solvent. This effect was investigated and a novel theoretical model developed that links experimental observations on nanoscale mechanical sensors. Simulations conducted in the presence of water show that hydrodynamic effects have to be taken into account in order to properly characterize the sensitivity of these sensors. This work, some of which was described in our previous report, has been published in the prestigious journal *Physical Review Letters*.

Functionalization: The process described above exposes the three surfaces of the silicon nanowire along the longitudinal direction.. After the fabrication of the silicon nanowire and the gold electrodes and gates, a protective layer of polymethylmethacrylate (PMMA) is spun on the surface and only the silicon nanowire is exposed by a secondary *e*-beam exposure, while the device floor of oxide remains covered. This process allows exposure of only the silicon nanowire to air/solution. The functionalization of the nanowire surface is done by the application of a 2% APTES solution of methanol for 3 hours. After multiple rinsing of the device by methanol, the device is dried by nitrogen gas and baked at 80°C in an oven for 10 minutes. fabrication method used. Characterization of surface functionalization was tested using a fluorescently labeled streptavidin-biotin system, and independently by an Atomic Force Microscope. Preliminary results of Atomic Force Microscopy and Fluorescence were described in our previous report.

Nanosensor arrays: Figure 3 - Figure 5 describe a key research accomplishment of our report – the fabrication of an array of nanosensors, and their characterization using the Field-effect. Figure shows an electron microscope image of the array. Shown in the figure are 9 electrodes, coated with gold, and a tenth electrode used as a reference potential to locally set the potential of the solution of buffer and protein used for electrical measurements. Bridging the electrodes are sets of nanowires. To show the flexibility of the fabrication process, several

different structures have been fabricated on the same device. The device shown has wires of different widths, from 200 nm wide to 300 nm wide, to a single silicon strip that is 10 μm wide as a control. The ability to control geometry to this level may be compared to conventional gene chip optical arrays, in which a single 20 μm pixel would span the dimension of about 40 nanosensors shown here.

As a test, the nanosensors were used to sense hydrogen ion concentration (pH). The estimated sample volume sensed is about 1 femtoliter. The effect of pH can be mimicked by changing the gate voltage, increasing sensitivity and control by electrically “tuning” the electric field near the nanosensor past the pK value of a selected biomarker target group. This ability of using gate voltage to increase sensitivity is shown in the last figure. Increasing the gate voltage increases the differential conductance change as the ion concentration is changed. Conversely, the gate voltage can be used to “tune” the effective local ion concentration near the nanosensor, using a Field-Effect principle. This suggests that a suitable gate voltage can be used effectively to change “local pH” in a few femtoliters of solvent that are near the nanosensor. By changing the local proton ion concentration past the nominal chosen pK value, it is possible to control simply by using gate voltages the ability of a biomarker to bind or not, thus allowing for selective coating of the nanowire with specific antibodies or peptides. This aspect will be investigated using specific breast cancer biomarkers. Figure 4 and Figure 5 (next page) show some current-voltage characteristics (I-V) characteristics of the nanosensor array. Operation of the BioFET in the saturation mode appears to provide the best combination of sensitivity and reproducibility. While measurements on the 200 nm wide nanosensors are the most sensitive, the 300 nm wide nanosensors are more stable and provide a margin of reproducibility that is important for an actual device to be used in clinical applications. We propose to work with 300 nm wide devices for studying breast cancer biomarkers.

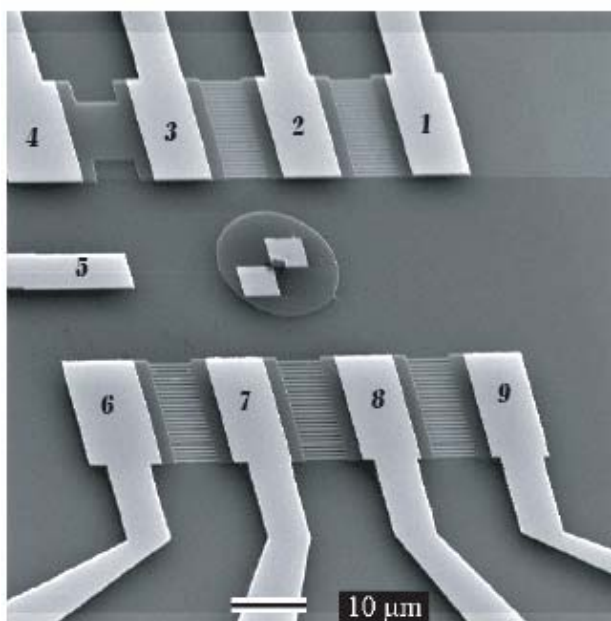
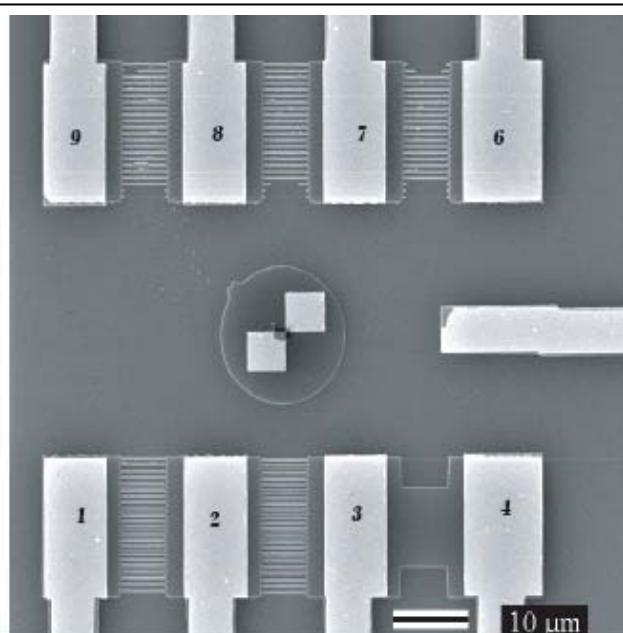


Figure 3. Fabrication of an array of nanosensors. (Top) Electron micrograph of nanosensor arrays, containing 92 individual elements. Also shown is a control silicon strip, connected by electrodes 3 and 4. The scale bar of 10 microns shows the excellent spatial resolution obtained in nanofabrication.

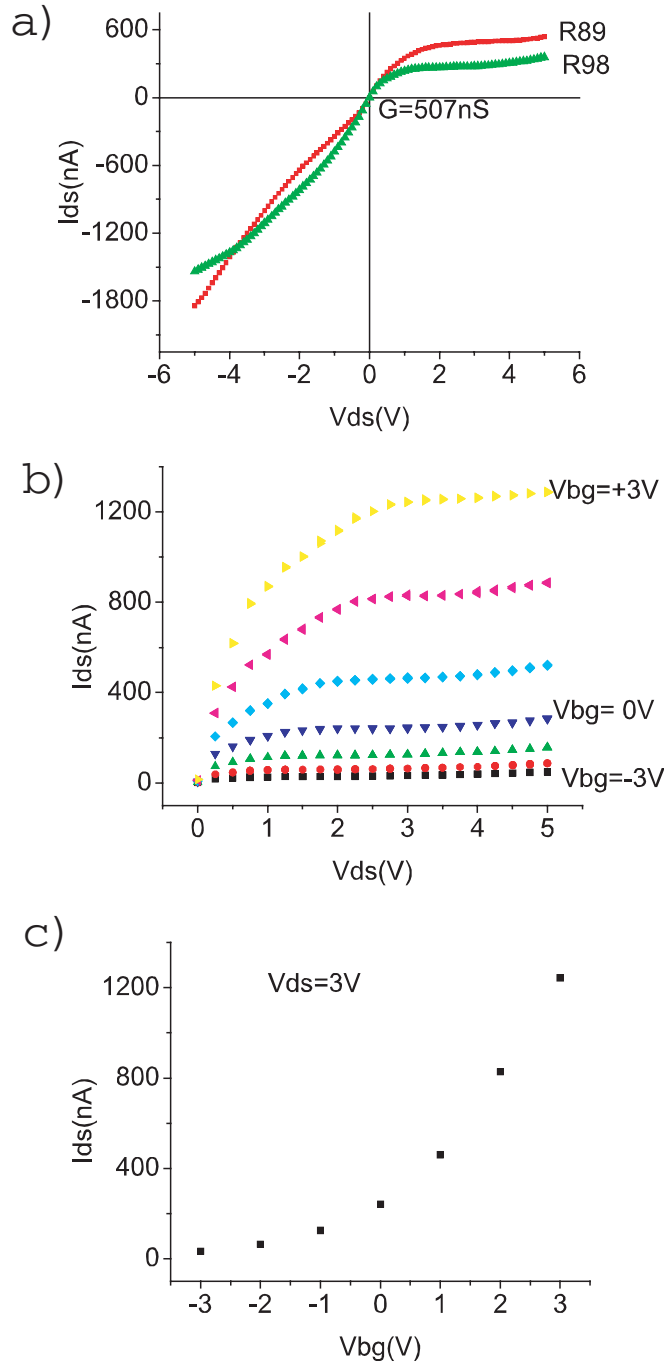


Figure 4. *I-V* results of 19 silicon wires with 200nm width in parallel (100nm thick, 6 μ m long), data was taken in air.

- I-V* characteristic of the sample (R89, R98 represent data taken in two scanning directions. The conductance was estimated to be 507nS at zero bias)
- I-V* results of the sample with different back gate voltage (-3V to 3V from bottom to the top, step is 1V)
- Source drain current at 3V bias (saturated) vs. back gate voltage.

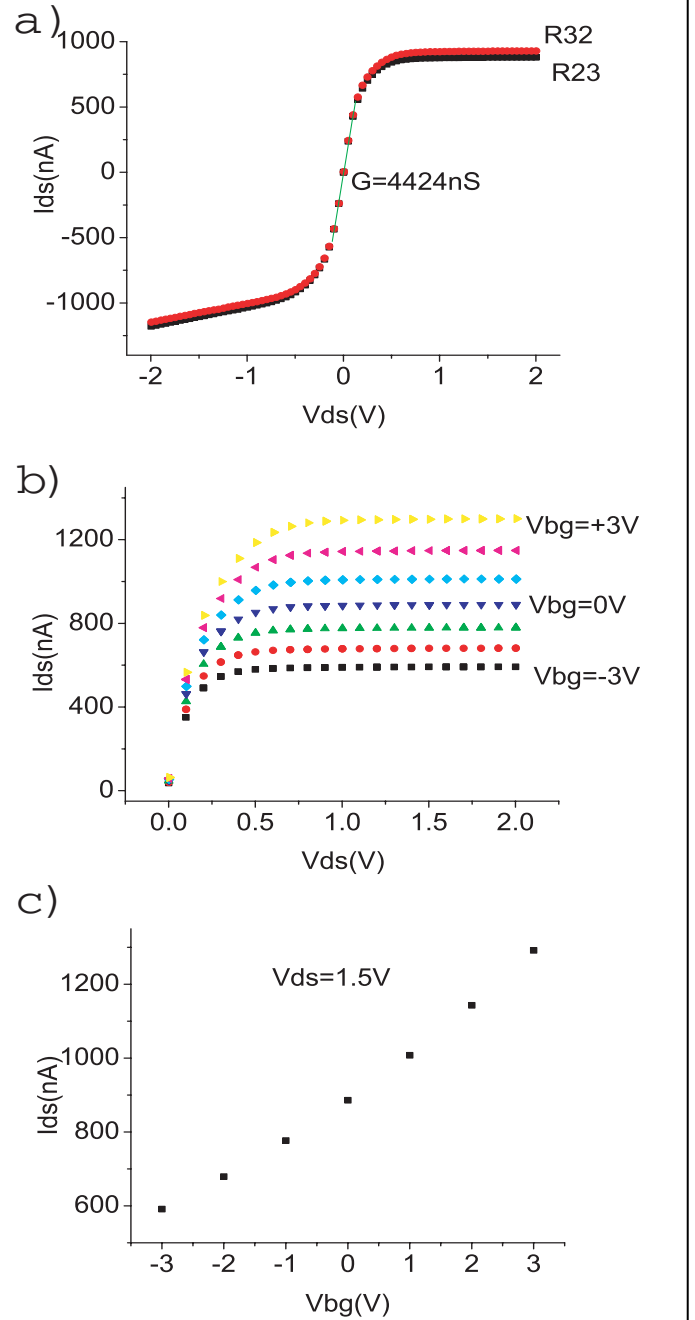
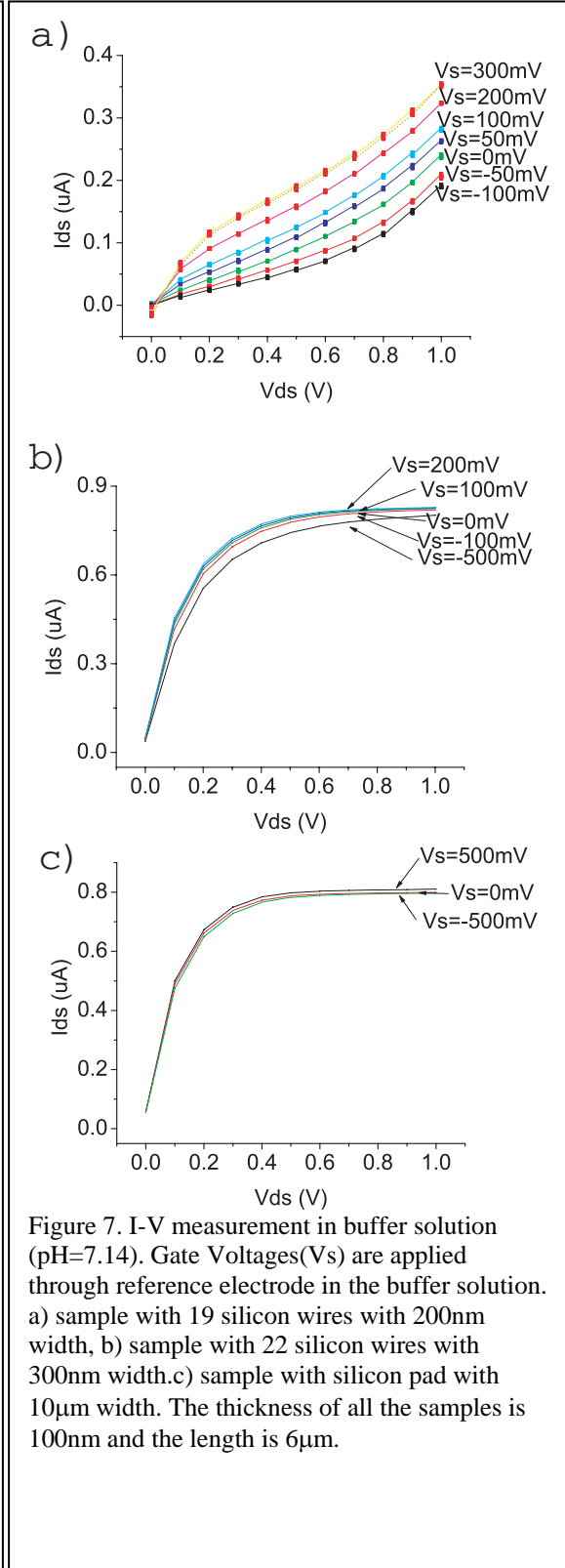
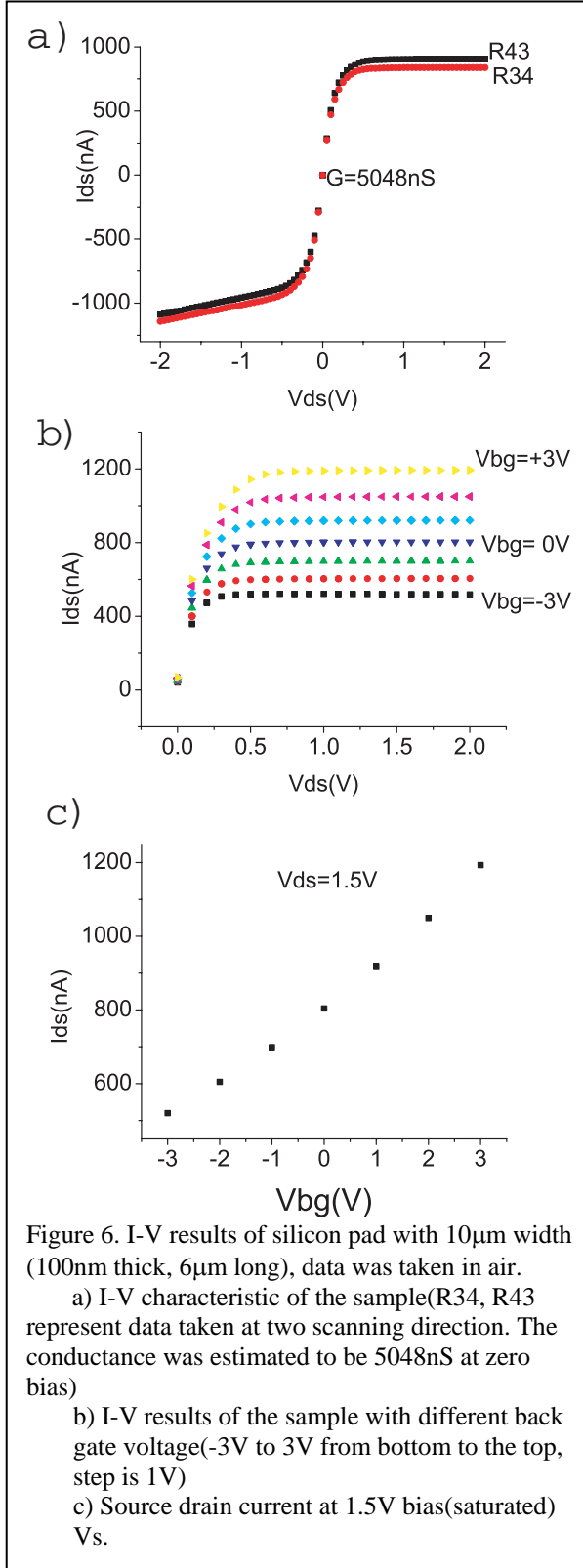


Figure 5. *I-V* results of 22 silicon wires with 300nm width in parallel (100nm thick, 6 μ m long), data was taken in air.

- I-V* characteristic of the sample (R23, R32 represent data taken at two scanning direction. The conductance was estimated to be 4424nS at zero bias)
- I-V* results of the sample with different back gate voltage (-3V to 3V from bottom to the top, step is 1V)
- Source drain current at 1.5V bias (saturated) vs. back gate voltage.

Figure 6 and Figure 7 show the operation of the nanosensors and control silicon pads treated as as a gated Field effect transistor (BioFET). Operation in the saturation mode appears to provide the most reliable operation while maintaining sensitivity.



Conclusions

To summarize the electrical conductance studies on the nanosensor arrays: we have shown that it is possible to fabricate an array of nanosensors and to operate them in a Field-effect mode. While there is scope for further refinement of the electrical studies on the devices, and investigation of the physics of the fabricated devices, the data are sufficiently encouraging to start testing the devices on biomarkers derived from patient tissue. We plan to initiate the studies as soon as pending approval is granted.